THE INTRACUTANEOUS 'TYPHOIDIN' REACTION *

III. THE RELATION OF CUTANEOUS HYPERSENSITIVENESS TO EXPERIMENTAL IMMUNITY AND INFECTION

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The conception that cutaneous hypersensitiveness is indicative of resistance to an infection, is not new. Basing his views on studies of tetanus and diphtheria toxin, von Behring[1] expressed the opinion that hypersusceptibility to toxin meant an increased defensive activity on the part of the body against the specific microorganisms. Moreover, extensive investigations by numerous workers, Römer,[2] Calmette,[3] Krause,[4] and others, on the relation of tuberculin hypersensitiveness to immunity in tuberculosis, demonstrated that sheep and also guinea-pigs protected against experimental infection with the tubercle bacillus possess, as a rule, a high cutaneous or general hypersensitiveness to tuberculin. Observations made by Römer[5] as early as 1903 and, later, by one of us on a large number of bovovaccinated cattle in Pennsylvania demonstrated, however, apparent resistance to tuberculous infection without tuberculin hypersusceptibility. Furthermore, Krause[4] maintained that sensitization of nontuberculous guinea-pigs with tubercle protein does not alter their resistance to experimental tuberculous infection. Marked tuberculin hypersensitiveness in man and cattle has, thus far, in the majority of cases been considered a sign of progressive tuberculous infection; if the disease subsides, the hypersensitiveness gradually falls to a low level.

In the light of our discussion of the nature of the typhoidin skin reaction and the conclusion reached that it probably indicates sensitization to some complex protein, it is quite evident that cutaneous hypersensitiveness cannot very well be accepted as an index of immunity.

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[1] Einführung i. d. Lehre v. d. Bekämpfung der Infektionskrankheiten, 1912, pp. 141, 353.
[2] Handbuch d. Technik u. Methodik der Immunitätsforschung, 1908, 1, p. 932.
[3] Compt. rend. Soc. de biol., 1910, 68, p. 48.
[4] Jour. Med. Research, 1911, 24, p. 399.
[5] Handb. d. Technik u. Methodik der Immunitätsforschung, 1908, 1, p. 958, and Report of the Eighth International Veterinary Congress, Budapest, 1905, 1, p. 406.

It is possible that hypersensitiveness parallels the defensive activity of the body against the microorganism, but, as numerous observations have convinced us, absence of immunity certainly is not always associated with an absence of hypersensitiveness. Prolonged careful experiments are necessary to determine the interrelation of both conditions, and it is therefore with considerable hesitancy that we present in the following paragraphs some experiments which were mainly undertaken to show how difficult it is to conduct studies on typhoid immunity on rabbits. The technic of intravenous inoculation of many millions of typhoid organisms, which has to be chosen in order to produce conclusive results, is certainly a very drastic method of artificial infection, and is quite out of proportion to the natural mode of infection in man.

EXPERIMENT-GROUPS 1 AND 2

THE RELATION OF THE TYPHOIDIN TEST TO IMMUNITY

Two groups of experiments were carried out to determine the relation of the various skin reactions to the immunity of rabbits against subsequent infections by intravenous injections of large amounts of a recently isolated strain of B. typhosus, or by the injection of twice to three times the lethal dose of a fowl-typhoid culture. Most of the rabbits used in these groups were immunized with various types of vaccines, and were respectively tested with different kinds of typhoidin preparations.

Series 1.—Twelve rabbits (300 to 311 inclusive) had been immunized with 4 different kinds of vaccines. Rabbits 300, 301, 302, 309, 310, and 311 had each received 3 injections of an army type of vaccine; Rabbits 303, 304, and 305 had been treated with a sensitized—and Rabbits 306, 307, and 308 with a polyvalent typhoid-paratyphoid-A and -B vaccine. Every animal had received the amounts of vaccine ordinarily used for the prophylactic immunization of man, and at the customary intervals. The army and polyvalent types of vaccines had been given at 10-day intervals, and the sensitized vaccine on alternate days.

On November 1, or 34 and 46 days, respectively, after the last injection, the rabbits were tested with typhoidin and fowl-typhoidin preparations. These glycerin extracts had been used previously and found suitable in dosage of 0.001 gm. Our experience with this test is summarized in Table 1.

Immediately after this test, every rabbit was inoculated intravenously with one-thirtieth slant of a fowl-typhoid culture. The 6 rabbits which resisted this infection were tested again on March 15—105 days after the first test, or 139 and 151 days, respectively, after the last injection—with various typhoidin and B.-dysenteriae extracts.

The preparations used had the following antigenic values: Typhoidin B, an extract of a 24-day-old glycerin potato-broth culture, contained 6.6 extract units (e.u.) per 0.001 gm. Typhoidin 3, the same preparation as was used in Group 1 (Table 3), had, at the time of test, only 33.3 e.u. per 0.001 gm. No

determinations of antigenic value were made on the previously tested glycerin Witte's-peptone-broth extract of B. dysenteriae Shiga (Do).

On April 13, 210 and 198 days, respectively, after the last injection of typhoid vaccine, or 162 days after the immunity test with fowl-typhoid bacilli, the 6 rabbits (301, 304, 307, 308, 309, and 311) were each inoculated intravenously with one-fourth slant of a 24-hour-old rabbit-blood agar culture of a recently isolated strain of B. typhosus (H-125). An interval of about 28 days after the typhoidin test was chosen to avoid any influence of the antigen on the antibody-production in the rabbits to be tested. Five normal rabbits (641 to 645) served as controls.

This extensive experiment is summarized in Table 1. In the first typhoidin tests there were positive reactions in all the vaccinated rabbits, with the exception of 306 and 307, which had been injected with a trivalent typhoid-paratyphoid vaccine. All the skin reactions were distinct, but fluctuations in the size of the areola and the degree of induration were noticed among the rabbits of one and the same lot. Vaccines A and B provoked the best reactions; those to Vaccine D were fair in some animals, the areola being not much larger than in the control rabbits. No parallelism between intensity of reaction and amount of serum agglutinins could be demonstrated. Of interest, however, was the striking parallelism exhibited in the degree of skin reaction of the same rabbit (see 300, 302, 303, 304, etc.) to fowl typhoidin. Again, in 306 and 307 reactions were absent, and in 305 and 308 they were marked and persisted well up to the 24th hour. The areolae were found to be very slight or indistinct in the rabbits which had failed to respond distinctly to typhoidin (310 and 311). Unfortunately, at the time these tests were carried out we did not consider it necessary to make 48-hour readings, but it will be noticed in Table 1 that the typical reactions either were the same at the 24-hour reading, or had increased in intensity over those of the 16th hour. It may be assumed, therefore, that the reactions would have persisted up to the 48th hour. In the amount of 1 mg. chosen for these tests, typhoidin and fowl-typhoidin extracts were only slightly irritant to the skin of normal rabbits.

The immunity tests of the rabbits protected with Vaccine A showed that the 2 rabbits (300 and 302) which gave the best skin reactions, succumbed to infection with fowl-typhoid organisms. In the group of rabbits injected with Vaccine B., one animal died from the infection, while another (305) had become so nonresistant as a result of the intoxication that it fell an easy prey to a secondary infection with B. cuniculisepticus. Both animals had given good positive skin reactions which differed slightly from those of Rabbit 304, which resisted

Rabbit	Immunization	Last Injection	Previous Test	Serologic Test March 28		Hours	Typhoidin 16 1:50	Typhoidin 16 1:100	Control Powder 1:10	Paratyphoidin B 1:50	B. Coli 16 1:50	Tuberculin 1:50	Ho
				Agglutination	Complement-Fixation								
340	A. vaccine 3 injections	Nov. 30	Jan. 23	1:400	0.05 A* 0.005 S	16	1.9 x 1.9	1.7 x 1.7	Spot	2 x 2	2.9 x 2.9	Spot	1
						24	2.7 x 2.3	1.5 x 1.5	2.4 x 2.4	2.4 x 2.4	2.7 x 2.7	2.1 x 2.1	2
						48	2.5 x 2.5	2 x 2	2 x 2, not raised	2.7 x 2.7	2.3 x 2.3	Spot	4
341	A. vaccine	Nov. 30	Jan. 23	1:600— — —	0.05 A	16	2.3 x 2.3, R. I.	Diffuse red area	Spot	2.9 x 2.9, R. I.	2.1 x 2.1, Diff. and Ind.	Red spot	
	3 injections			1:1000—	0.05 S	24	2.4 x 2.4	1.4 x 1.4	Spot	2.1 x 2.1, R. I.	2.5 x 2.5, Diff. and Ind.	Red spot	
						48	2.8 x 2.8	1.4 x 1.4	1.4 x 1.4	2.4 x 2.4, R. I.	2.9 x 2.9, R. I.	1.3 x 1.3	
						72	1.7 x 1.7	N. P.	Papule	N. P.	2.8 x 2.8	Red spot	
342	B. vaccine 3 injections	Nov. 16	Jan. 23	1:100— —	0.05 A 0.05 S	16	1.9 x 1.9, R. I.	1.3 x 1.3	Spot	1.6 x 1.6, R. I.	1.3 x 1.3, R. I.	Spot	1
						24	1.6 x 1.6, R. I.	1.3 x 1.3	Spot	1 x 1	1.3 x 1.3	Spot	2
						48	1.7 x 1.7, R. I.	0.9 x 0.9	N. P.	1.1 x 1.1	1.5 x 1.5	Spot	4
						72	Spot	Spot	Red spot	Red spot	1.4 x 1.4, red area	Spot	
343	B. vaccine 3 injections	Nov. 16	Jan. 23	1:200— — — 1:400—	0.05 A 0.05 S	16	2.1 x 2.1	1.7 x 1.7	Red spot	1.8 x 1.8, I. R.	1.9 x 1.9, R. I.	1.8 x 1.8, R. I.	
						24	2.4 x 2.4	1.9 x 1.9	Red spot	2.3 x 2.3	1.8 x 1.8, R. I.	1.6 x 1.6, red	
						48	2.3 x 2.3	1.7 x 1.7	N. P.	2.1 x 2.1	3 x 3, R. I.	1 x 1, red	
						72	Red spot	Red spot	N. P.	N. P.	3 x 3, red area	N. P.	
344	B. vaccine 3 injections	Nov. 16	Jan. 23	1:200— — —	0.05 A 0.05 S	16	2 x 2	1.7 x 1.7	N. P.	N. P.	1.3 x 1.2, S. I.	0.8 x 0.8, S. I.	
						24	2.6 x 2.6	1.9 x 1.9	N. P.	Red spot	Diffuse area	Red spot	
						48	2.5 x 2.5	1.6 x 1.6	N. P.	1.4 x 1.4	3.4 x 3.4	1.5 x 1.5	
						72	Red spot	Red spot	N. P.	N. P.	2 x 2, red area	N. P.	
346	C. vaccine (polyvalent) 3 injections	Nov. 30	Jan. 23	1:200— — —	0.05 A <0.05 S	16	1.6 x 1.6, W. D.	1.1 x 1.1, R.I., good	Red spot	2.1 x 2.1, R. I.	1.5 x 1.5, R. I.	1 x,1, R. I.	1
						24	1.7 x 1.7, R. I.	0.9 x 0.9	Spot	1.9 x 1.9, R. I.	1.5 x 1.5, R. I.	1 x 1, R. I.	2
						48	1.6 x 1.6	09. x 0.9	0.4 x 0.4	1.8 x 1.8, R. I.	2 x 2, R. I.	0.8 x 0.8, R. I.	4
						72	Red spot	N. P.	N. P.	Red spot	2 x 2, R. I.	N. P.	
347	C. vaccine (polyvalent) 3 injections	Nov. 30	Jan. 23	1:600— — — 1:800—	0.05 A 0.05 S	16	2.1 x 2.1, W. D.	1.7 x 1.7	Spot	2.1 x 2.1, R. I.	2.4 x 2.4, R. I.	1.6 x 1.6, R. I.	
						24	2.6 x 2.4, W. D.	1.2 x 1.2	Spot	1.5 x 1.5, R. I.	2.1 x 2.1, R. I.	1.4 x 1.4, R. I.	
						48	2.4 x 2.4	0.5 x 0.5	Spot	2.1 x 2.1, R. I.	2.7 x 2.7, R. I.	0.9 x 0.9, R. I.	
						72	Red area	Red spot	N. P.	N. P.	2.5 x 2.5, R. I.	N. P.	
348	D. vaccine 3 injections	Nov. 30	Jan. 23	1:600 1:800— —	0.05 A 0.05 S	16	1.6 x 1.6, R. I.	Red spot	Red spot	1.5 x 1.5, R. I.	1.8 x 1.8, D. R.	1.6 x 1.6, R. I.	
						24	1.8 x 1.8	N. P.	N. P.	1.9 x 1.9	1.9 x 1.9	1.7 x 1.7	
						48	2.1 x 2.1, R. I.	N. P.	N. P.	2.8 x 2.8, R. I.	1.8 x 1.8, red area	0.9 x 0.9	
						72	Red spot	N. P.	N. P.	Red spot	Red ind.	N. P.	
349	D. vaccine 3 injections	Nov. 30	Jan. 23	1:100 1:200— —	0.05 A 0.05 S	16	Red spot	Diffusely red spot	N. P.	1.9 x 1.9, D. I.	Diffuse area	Diffuse area	1
						48	1.9 x 1.9	1.6 x 1.6	N. P.	2.1 x 2.1, R. I.	3.1 x 3.1, R. I.	0.5, red spot	2
						72	1.6 x 1.6	N. P.	N. P.	Red spot	Red area	N. P.	4
350	D. vaccine 3 injections	Nov. 30	Jan. 23	.....	.....	....	.....	.....	.....	.....	.....	.....	..
632	Control			Negative	0.1 (50%)	16	N. P.	0.6, nod. indur.	0.5, S. I.	Red spot	0.5, S. I. spot	N. P.	
						24	N. P.	0.2, nod. indur.	Spot	Red spot	0.5, S. I. spot	N. P.	
						48	N. P.	N. P.	N. P.	N. P.	2.2 x 1, red	N. P.	
						72	N. P.	N. P.	N. P.	1 x 1	2 x 2, R. I.	N. P.	
640	Control	.....	.....	.....	.....	....	.....	.....	.....	.....	.....	.....	..
655 656	Control 1/10 slant of fowl typhoid	.....	.....	.....	.....	....	.....	.....	.....	.....	.....	.....	..

* A = antigen titration. S = serum titration.

XPERIMENT-GROUP 1

Serologic Tests		Hours	Typhoidin B 1:20	Typhoidin B 1:400	Control 1:10	Typhoidin 3 1:100	Control 3C 1:10	Dysentery Do. 1:400	Immunity Test for Typhoid	Result
gglu-ina-ion	Complement-Fixation									
		16	1.5 x 1.5, R. I.	2.2 x 2.2, I. R.	Red spot	1.8 x 1.8, R. I.	0.3 x 0.3, R. I.	0.2, R. I.	April 13, ½ blood-agar slant. (H-125) I.V.*	Carrier (gallbladder)
		24	2.4 x 2.4, R. I.	1.4 x 1.4, W. D.	Red spot	1.7 x 1.7, R. I.	Red spot	Red spot		
		48	2.3 x 2.3, good	1 x 1, S. I.	N. P.	1.4 x 1.4, R. I., good	Red spot	0.9 x 0.9		
ch 14, :100	0.01 (typhoid) 0.03 (fowl typhoid)	16	1.6 x 1.6	Red spot	Red spot	D. inf. 1.6 x 1.6	Red spot	Red spot	April 13, ½ blood-agar slant. (H-125) I.V.*	Carrier (gallbladder)
		24	1.4 x 1.4, W. D.	Red spot	Red spot	D. inf. 1.6 x 1.6	Red spot	Red spot		
		48	1.5 x 1.5, S. I.	N. P.	N. P.	D. inf. 2.7 x 2	Red spot	Red spot		
:400	0.01 (typhoid) 0.05 (fowl typhoid)	16	—	Red spot	Red spot	1.8 x 1.8, I. R.	Red spot (0.5 x 0.5)	N. P.	April 13, ½ blood-agar slant. (H-125) I.V.*	Died 4 days after injection from typhoid septicemia
		24	—	Red spot	N. P.	1.7 x 1.7, Sl. redness	Red spot	Red spot, 0.1 x 0.1		
		48	—	N. P.	N. P.	1.2 x 1.2, nod., W. D.	Red spot	Red spot		
:1000 :4000	0.01 (typhoid) 0.03 (fowl typhoid)	16	—	1.9 x 1.9, R. I.	Red spot	1.4 x 1.4	Red spot	Red spot	April 13, ½ blood-agar slant. (H-125) I.V.*	Died 3 days later from typhoid septicemia
		24	—	1.8 x 1.8, R. I.	Red spot	2 x 2, diffuse	Red spot	Slightly ind. area		
		48	—	N. P.	N. P.	1.6 x 1.6, R. I.	N. P.	N. P.		
:200	0.01 (typhoid) 0.03 (fowl typhoid)	16	—	Red spot	N. P.	2.2 x 2.2, W. D.	1.3 x 1.3, R. I., W. D.	Red spot	April 13, ½ blood-agar slant. (H-125) I.V.*	Resisted infection
		24	—	Red spot	Red spot	1.8 x 1.8, red, W. D.	1.2 x 1.2, R. I., W. D.	N. P.		
		48	—	N. P.		1.8 x 1.8, red, W. D.	N. P.	N. P.		
:100	0.03, 0.01 (typhoid) 0.05, 0.1 (fowl typhoid)	16	—	Red spot	Red spot	2.5 x 2.5, D. I.	1 x 1, D. I.	Red spot	April 18, ½ blood-agar slant. (H-125) I.V.	Carrier (gallbladder)
		24	—	Red spot	Red spot	2.5 x 2.5, D. I.	1 x 1, D. I.	Red spot		
		48	—	N. P.	N. P.	Diffuse red infiltration	N. P.	N. P.		
:20	0.1 (typhoid)	16	Red spot 0.2	Red spot	N. P.	Red spot	Red spot, 0.1 x 0.1	N. P.		Bled to death March 21, 1916
		24	Red spot	Red spot	N. P.	Red spot	Red spot	N. P.		
		48	Red spot	Red spot	N. P.	N. P.	N. P.	N. P.		

Controls 641, 642, 643, 644, and 645 were inoculated at the same time, and killed 1 month later. Three (641, 642, and 645), or 60%, me carriers (liver and bile)
mmune rabbits: Five, or 83.3%, either became carriers or succumbed to acute infection

Rabbit	Immunization	Last Injection	Hours	Serologic Tests		Typhoidin 3 1:100	Fowl-Typhoidin 3 1:100	Control 1:100	Results of Immunity Tests	
				Agglutination	Complement-Fixation					
300	Vaccine A 3 injections of 0.5, 1, 1 c.c.	Sept. 28, 1915	16	1:6400	—	2.8 x 2.8, R. I.	2 x 2, R. I.	N. P.	Tested Nov. 3 with 1/30 slant of fowl typhoid (2 lethal doses)	Died Nov. 9, 19 from fowl-t phoid infectio gallbladder ca rier
			24			2 x 2.8, R. I.	1.9 x 1.9, R. I.	N. P.		
301			16	1:6400	—	1.8 x 1.8, R. I.	1.8 x 1.8, R. I.	N. P.	Controls died in 2½ days	Resisted infecti with fowl t phoid
			24			1.5 x 2.1, R. I.	0.8 x 0.8, R. I.	N. P.		
302			16	1:6400	—	2.2 x 2.2, W. D.	2 x 2, S. R.	N. P.	Controls died in 2½ days	Died Nov. 15 fro fowl-typhoid fection
			24			1.6 x 1.6	1.6 x 1.6, S. R.	N. P.		
303	Sensitized vaccine-sediment B 1 c.c., 1 c.c., 1 c.c.	Sept. 16, 1915	16	1:640	—	1.9 x 1.9	1.6 x 1.6	N. P.	Controls died in 2½ days	Died Nov. 14 fro fowl-typhoid fection; ga bladder carrie
			24			2.2 x 2.2, R. I.	2 x 2, R. I.	N. P.		
304			16	1:640	—	2.4 x 2.4, R. I.	2.5 x 2.5	N. P.	Controls died in 2½ days	Resisted fowl-t phoid infectio
			24			2.2 x 2.2, R. I.	2.3 x 2.3, W. R.	N. P.		
305			16	1:640	—	2.3 x 2.3, R. I.	2 x 2, W. D.	N. P.	Controls died in 2½ days	Died Nov. 20 fro secondary infe tion (B. cunic septicus) caus by fowl typho
			24			2.1 x 2.1, W. D.	2.4 x 2.4	N. P.		
306	Vaccine C	Sept. 28, 1916	16	1:3200	—	0.4 x 0.4	0.2 x 0.2	N. P.	Controls died in 2½ days	Died Nov. 18 fro fowl-typhoid fection
			24			0.2 x 0.2	0.3 x 0.3	N. P.		
307			16	1:6400	—	0.5 x 0.5, D. I. R.	0.2 x 0.2	N. P.	Controls died in 2½ days	Resisted fowl-t phoid infectio
			24			0.2 x 0.2	0.3 x 0.3	N. P.		
308			16	1:3200	—	1.1 x 1.1	1.9 x 1.9	N. P.	Controls died in 2½ days	Resisted fowl-t phoid infectio
			24			1.9 x 1.9, R. I.	2.3 x 2.3, R. I.	N. P.		
309	Vaccine D	Sept. 28, 1916	16	1:3200		2.6 x 2.6, W.D., red	1.5 x 1.5, R. I.	N. P.	Controls died in 2½ days	Resisted fowl-t phoid infectio
			24			2.9 x 2.9	2.3 x 2.3	N. P.		
310			16	1:1600		0.3 x 0.5	0.6 x 0.6	N. P.	Controls died in 2½ days	Died Jan. 15 fro secondary infe tion
			24			1.4 x 1.4	0.3 x 0.3, red area	N. P.		
311			16	1:3200		1 x 1	1.2 x 1.2	N. P.	Controls died in 2½ days	Resisted fowl-t phoid infectio
			24			0.6 x 0.6	0.6 x 0.6	N. P.		
572	Controls		16			0.2 x 0.2	0.4 x 0.4, indur. nodule	N. P.	Controls died in 2½ days	
			24			0.3 x 0.3	0.2 x 0.2	N. P.		
573			16			1 x 1	0.4 x 0.4	N. P.		
			24			0.5 x 0.5	0.2 x 0.2	N. P.		
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KEY TO ALL TABLES

W. D. = well-defined areola
R. I. = deep purplish, indurated area
I. R. = indurated red area
S. I. = very slight induration
D. O. = diffuse edema
S. O. = slight edema
D. R. I. = diffusely indurated and red
D. R. area or D. R. A. = diffuse red blush
S. spot = small spot
N. P. = needle puncture, traumatic reaction

PERIMENT-GROUP 2

owl-Typoidin 4 1:50	Fowl-Typhoidin 4 1:100	Control Powder	Staphylococcin 1:50	Paratyphoidin-B3 1:50	Dysentery Do. 1:50	Immunity Test for Fowl Typhoid	Result	Immunity Test for Typhoid	Result
4 x 2.4	1.8 x 1.8	N. P.	2.8 x 2.8, I. R.	1.2 x 1.2	1.4 x 1.4, R. I.	April 18, 1/10 slant of fowl-typhoid bacilli (3 times lethal dose)	Died from fowl typhoid April 21		
2 x 2.2	1.4 x 1.4	Red spot	2.5 x 2.5	1.5 x 1.5	1.6 x 1.6				
1 x 2.1	1.4 x 1.4	N. P.	2.2 x 2.2	0.6 x 0.6	1.3 x 1.3				
								April 27, ½ blood-agar slant of B. typhosus H-125, intravenously*	Resisted infection
5 x 2.5	1.6 x 1.6	N. P.	N. P.	0.2 x 0.2	N. P.	April 18, 1/10 slant of fowl-typhoid bacilli	Died from fowl typhoid April 20		
9 x 1.9	1.6 x 1.6	N. P.	N. P.	1.4 x 1.4	N. P.				
5 x 1.5	1.3 x 1.3	N. P.	Red spot	Red spot	N. P.				
								April 27, ½ blood-agar slant of B. typhosus H-125, intravenously*	Died April 29 from typhoid septicemia
								April 27, ½ blood-agar slant of B. typhosus H-125, intravenously†	Resisted infection
4 x 2.4, Diffuse	1.6 x 1.6, Diffuse	Red spot	N. P.	Red spot	Red spot	April 18, 1/10 slant of fowl-typhoid bacilli	Died from fowl typhoid April 21		
8 x 2.6	1.7 x 1.7	N. P.	N. P.	1.6 x 1.6	1.8 x 1.8				
2 x 2	1.5 x 1.5	N. P.	N. P.	1.4 x 1.4	2.1 x 2.1				
								April 27, ½ blood-agar slant of B. typhosus H-125, intravenously*	Carrier (liver) on May 26
								April 27, ½ blood-agar slant of B. typhosus H-125, intravenously*	Carrier (gallbladder) on May 26
2 x 2.2	1.8 x 1.8	N. P.	N. P.	N. P.	0.5 x 0.5, R. I.	April 18, 1/10 slant of fowl-typhoid bacilli	Died from fowl typhoid April 20		
8 x 1.8	1.6 x 1.6	N. P.	N. P.	N. P.	1.5 x 1.5, R. I.				
7 x 1.7	1.2 x 1.2	N. P.	N. P.	N. P.	1 x 1, diff. swelling				
.....	.....	.....	.....	.....	.....		Died March 30 from intracurrent disease		
.....	.....	.....	.....	.....	.....	.....	.....	April 27, ½ blood-agar slant of B. typhosus H-125, intravenously*	Died from typhoid septicemia May 1
x 0.2, d spot	N. P.	N. P.	N. P.	0.5 x 0.5, R. I.	N. P.				
. P.	N. P.	N. P.	N. P.	N. P.	N. P.				
. P.	N. P.	N. P.	N. P.	N. P.	N. P.				
.....	.....	.....	.....	.....	.....	April 18, 1/10 slant of fowl-typhoid bacilli	Died April 19, and 20		

† Controls in immunity test, 669, 670, 632, 640, were inoculated at the same time and examined postmortem 1 month later. One rabbit (632), or 25%, died from typhoid septicemia.
Immune rabbits: 3, or 60%, died or became carriers.

the infection. In Vaccine-group C the results are even more contradictory to the conception that cutaneous hypersensitiveness is indicative of immunity; 1 rabbit (306) with a negative skin reaction died acutely from the infection, while the mate (307), which showed very little sensitization, together with an animal (308) which reacted positively, resisted the infection.

Similar conditions developed in Vaccine-lot D, in which a non-reacting rabbit overcame the immediate effect of the fowl-typhoid bacillus, but died later of a secondary infection. Of the 2 rabbits which resisted the infection, 1 gave a slight reaction with fowl typhoidin (311). These observations are in harmony with the work of Theobald Smith and Ten Broeck,[6] who have presented evidence that the human-typhoid bacillus tends to protect animals against the toxins of the fowl-typhoid bacillus, a finding which we were able to confirm in previous experiments. It was for this reason that we chose the fowl-typhoid bacillus as a test organism.

In comparison with the control rabbits (469, 470, 471, and 537), all of which succumbed to the intoxication and infection in from 2½ to 3 days, the typhoid-vaccinated rabbits showed a certain degree of resistance to the infection. The heart-blood cultures contained few or no organisms, while those of the controls were strongly positive.

The resistance of some of the rabbits to fowl-typhoid bacilli was not indicated by exceptionally marked skin reactions. Rabbits with small and doubtful skin reactions showed as much resistance as those which had extensive cutaneous areolae. It is therefore impossible to conclude from these tests that any relationship exists between the immunity of an animal to an overwhelming infection with the fowl-typhoid bacillus and the presence, or absence, or intensity, of a skin reaction produced by the intracutaneous inoculation of a glycerin extract of this particular test organism.

The remaining 6, apparently healthy, rabbits, when tested several months later with 2 types of typhoidin, responded well with 1 preparation. Only 1 rabbit (301) gave, with 0.00025 gm. of typhoidin B, an areola lasting longer than 24 hours. The positive reactions with Typhoidin 3 were well defined and lasted for at least 48 hours; some of the reactions were very intense and were indicated by diffuse edema, which often made it impossible to read the diameter of the areola. In the dosage of 0.00025 gm. chosen, the dysentery extract was inactive.

[6] Jour. Med. Research, 1914-1915, 31, p. 545.

The sera of most of the rabbits had low agglutinin and complement-fixing antibody content; only 1 rabbit (308) showed an agglutination of from 1:1000 to 1:4000. On subsequent immunity tests, Rabbits 307 and 308 died acutely from typhoid intoxications, Rabbits 301, 304, and 311 became carriers (gall-bladder), and only 1, Rabbit 309, resisted the infection.

If the skin reactions of these animals are considered as indices of immunity, it is well to record that Rabbit 309, in the first 24 hours, gave, so far as we could discern, a better reaction than all of the others, yet the skin of this rabbit reacted also to the control powder 3 c. Therefore, it is not unlikely that this rabbit possessed a hypersensitiveness, individually, more marked than in the other rabbits. That this single positive result has little value is further shown by a comparison of the other rabbits. It is also clear from Table 1 that all the rabbits which gave similarly strong reactions on the first and second tests either became carriers, or, as in the case of Rabbits 307 and 308, did not even resist the acute intoxication. In this connection it may be well to call attention to the fact that of 5 normal rabbis (641 to 645) which served as controls for the immunity tests, not one succumbed to the intoxication, and only 3, or 60%, became carriers. Five immune rabbits, or 83%, became carriers or succumbed to the infection.

We are unable to produce evidence that a positive skin test permits of the conclusion that a rabbit will resist a subsequent injection of a large dose of living typhoid bacilli (6000 millions). Data collected from other experiments which will be published in the near future, indicate that the typhoid-carrier stage in rabbits is strongly influenced by the existing serum immune bodies of the animal, and by other factors — biliary passages, bile, etc. — which have probably nothing to do with the cutaneous hypersensitiveness to typho- or bacterial proteins.

Series 2.—Ten rabbits (340, 341, 342, 343, 344, 346, 347, 348, 349, 350) were immunized with the same types of vaccines as were used in Series 1. Only 2 rabbits (340 and 341) were inoculated with Vaccine A; 3 (342, 343, and 344) with Vaccine B; 2 (346 and 347) with Vaccine C; and 3 (348, 349, and 350) with Vaccine D.

On January 28, 59 and 73 days, respectively, after the last injection, these rabbits were tested with Typhoidin 3 and Fowl-typhoidin 3. The results of this test have been discussed in Group 1 (Table 3, 1st paper).

On March 30, 121 days and 135 days, respectively, after the last injection, the animals were tested again with various typhoidin, paratyphoidin-B, tuberculin, and B.-coli extracts; on April 10, 1 rabbit of each lot was also tested a

second time with fowl-typhoidin, paratyphoidin-B, staphylococcus, and dysentery (Do) extracts.

The glycerin extracts used—with the exception of the tuberculin—were 1-month-old potato-broth cultures, concentrated and precipitated as usual. Typhoidin 16 had an antigenic value of 5 e.u. per milligram. No determinations were made for the paratyphoidin-B and B.-coli extracts. The fowl-typhoidin 4 and paratyphoidin B have been described in Group 2 (Table 3, 2nd paper).

These rabbits were infected intravenously with fowl typhoid bacilli on April 18, 139 and 153 days, respectively, after the last injection. The remaining 5 rabbits (341, 343, 344, 347, 348) were tested as usual with one-half slant of a rabbit-blood agar culture of a recently isolated strain of B. typhosus.

The results of these tests are shown in detail in Table 2. With typhoidin and Fowl-typhoidin 3, positive skin reactions were obtained in every rabbit. The degree and the persistence of the reaction, up to or after the 48th hour, were somewhat better in the A and C than in the B and D vaccine lots. That typhoidin in quantities smaller than 1 mg. calls forth a better response, was quite evident in the few tests in which each rabbit of the respective lots was tested with dilutions of the antigen. This observation confirms the findings of Force[7] that an army type of vaccine gives, in man, better sensitization than some of the other vaccine preparations.

The degree of the reaction apparently does not correspond with the amount of serum immune bodies demonstrable in the form of agglutinins and complement-fixing antibodies. This absence of parallelism is more pronounced in the second test, in which a low serum immunity is present together with a very marked and intense reaction. Every rabbit reacted to 0.02 gm. of Typhoidin 16, some of the reactions being strong and remaining so for 48 hours and longer. Several rabbits responded to the second typhoidin test as they did to the first. For example, in 342, 346, and 349 the areolae were less indurated and smaller, and faded more rapidly, than in the other rabbits. With 0.001 gm. of typhoidin, Rabbit 348 gave a negative reaction, and 342 and 346 gave very slight reactions.

These observations might be construed as having some significance, because neither animal resisted a subsequent fowl-typhoid infection, and the serum immune bodies were, on the average, low. One must recall, however, that these rabbits represent 3 different lots of vaccines of various composition, and that no definite conclusions can be drawn from so few observations.

The other glycerin extract chosen in this experiment, in dilution of 1 : 50, produced extensive nonspecific reactions. This was particularly

[7] Personal communication.

true of the B.-coli extract. Small nodules the size of peas, with small centers containing pus, persisted for from 4 to 6 days.

In our opinion these reactions were due to the leukotactic influence of the bacterial proteins, which were more concentrated in the B.-coli powder than in the other preparations on account of the heavier culture from which it was precipitated. The paratyphoidin-B extract gave skin reactions of varying degrees of intensity, but must be considered unreliable for the same reason.

The tuberculin preparation, prepared from a bovine strain, was filtered before being precipitated, and tested on numerous normal rabbits, with negative results. Rabbits 346, 347, and 348 gave skin reactions which, in regard to the diameter of the areolae and persistance of hyperemia and induration, must be regarded as suspicious. Several animals (343, 344, etc.) apparently showed a slight degree of cutaneous hypersensitiveness to a concentrated tuberculin, by small slightly indurated areolae. Two rabbits (342 and 349) which throughout the tests gave negative or doubtful typhoidin reactions, failed to develop skin reactions with tuberculin. The tuberculin reactions are the result of nonspecific cutaneous hypersensitiveness developing as a consequence of local sensitization with various extracts. This phase of the problem has been discussed in the previous paper.

The 4 rabbits (340, 342, 346, 348) which were tested with fowl typhoidin 10 days after the last general test, all reacted positively, the areolae being well-defined for 48 hours. The response to other glycerin extracts confirmed previous observations; that is, 342 failed to respond to the dysentery extract, which caused slight skin reactions in the remaining 3 rabbits. With the exception of 340, these rabbits all reacted to the staphylococcus extract. This particular animal was, apparently, hypersensitive to certain elements of the broth, for on previous tests it had reacted distinctly with the control powders. The result of this test is of very little value in the light of our subsequent findings on acquired local hypersensitiveness.

The 4 rabbits (340, 342, 346, 348) which reacted positively to fowl typhoidin in the first test (Table 3, 1st paper) and in the subsequent tests just discussed, did not resist an injection of 3 times the lethal dose of the fowl-typhoid bacillus. Until further experiments have been carried out, it is difficult for us to decide whether these animals died of the intoxication or of the infection. In the light of the state-

ments of Theobald Smith[6] and Bull,[8] the protection against fowl typhoid and typhoid, respectively, obtained by immunization with dead or living organisms is also directed against the toxins. We mention these points here because in recent studies on typhoid immunization the view has been expressed that the resistance against intoxication and that against infection are probably two separate functions in the defensive activity of the body. We are not as yet prepared to commit ourselves on this point, but record at this time some observations which show clearly that typhoid-immune rabbits are capable, to an extent, of handling the infection in the blood stream; for, at the time of death, the heart and portal blood of the 4 rabbits contained very few organisms (from 3 to 4 colonies per drop of blood), while the liver, spleen, and particularly the bile, contained a very large number of fowl-typhoid bacilli. In the control rabbits (655 and 656) just the reverse was the case; the blood was rich in bacteria, while the bile and organs contained comparatively few or no microorganisms.

The result of this immunity test with fowl-typhoid bacilli confirms the observations of Series 1 and supports the contention that a positive allergic skin reaction in a rabbit is not always proof that this type of animal is resistant to an infection or an intoxication with the organisms used as an antigen in the skin test.

Of the remaining 5 rabbits which represent the 4 types of vaccines, only 2 resisted an intravenous injection of a large dose of living typhoid organisms. Two animals became carriers, and 1 died suddenly of intoxication. The latter animal had developed a fair amount of serum immune bodies following the immunization, and had always responded well with a marked skin reaction. The same may be said of the other two rabbits, 347 and 348; they differed very little with regard to immune bodies and skin reactions from Rabbits 341 and 344, which resisted the infection. Rabbit 341 possessed more agglutinins and gave in the first typhoidin test a somewhat better reaction, but the differences were so slight that they are not worth further consideration (compare Table 3, 1st paper).

In comparison with the 4 control animals (632, 640, 669, 700), of which only 1, or 25%, succumbed to the infection, 3, or 60%, of the immune rabbits died or became carriers (gallbladder).

The conclusions derived from Series 1 are therefore supported by Series 2. Until we know more about the mechanism governing the

[8] Jour. Exper. Med., 1916, 24, p. 35.

typhoid-carrier stage and the immunity of the rabbit to typhoid, the only conclusion justifiable is that a positive allergic skin reaction in a rabbit does not indicate that this animal will resist a subsequent intravenous injection of living typhoid bacilli, or that the rabbit is so protected that it will not become a chronic carrier of bacilli in the gall-bladder or liver.

These experiments suggest, furthermore, that in rabbits cutaneous hypersensitiveness to various kinds of bacterial proteins may exist without the least degree of effectual resistance to the corresponding microorganism.

In the tables given in the preceding papers, we have presented the results of some of the immunity tests under the heading "Remarks." Whenever possible, each rabbit was tested after a certain time interval with the same strain which had been used for the immunization. From one-tenth to one-half agar slant was inoculated intravenously, and the result was determined by autopsies in from 1 week to 1 month, or even longer. For example, in Table 3 of our 1st paper, the outcome of the immunity tests in Rabbits 319, 325, 326, and 427, and so on, supports our views that a strongly positive skin reaction does not indicate that a particular animal is protected against an experimental infection. In the present communication it is impossible to call attention in detail to all of these interesting results, but we feel that the evidence collected corroborates fully the facts demonstrated in Experiment-group 1.

RELATION OF THE CUTANEOUS REACTION TO THE SERUM IMMUNE BODIES

It is not our intention to enter into a discussion of the relative values of the serum immune bodies as indices of immunity. The various tests for agglutinins and complement-fixing antibodies are so simply carried out that it is well to choose them as guides during the process of immunization and to consider them as an index of a response as well as presumptive evidence of the presence of other antibodies. The recent studies of Bull[9] on intravitam agglutination contributed important information relative to the value of agglutinins, and in connection with the typhoid-carrier problem in rabbits we have found that these immune bodies may play an interesting rôle.

[9] Jour. Exper. Med., 1915, 22, p. 475.

In analyzing the various serum findings reported in the different tables (also 1st and 2nd papers) we fail to find any parallelism between the agglutination titer, the amount of complement-fixing antibodies, and the intensity of the skin tests. Rabbits with a high agglutination titer frequently gave less pronounced cutaneous reactions than those with a low titer, and vice versa. In addition to this fact it was quite apparent that rabbits which were immunized against B. bipolaris produced an antiserum free from agglutinins or complement-fixing antibodies for B. typhosus, and yet showed intense cutaneous hypersensitiveness to this particular antigen. And again, rabbits which had lost their serum immune bodies would still give anaphylactic skin reactions.

Drawing conclusions from our observations on rabbits, we may state that no definite relationship exists between agglutinins and complement-fixing antibodies and cutaneous hypersensitiveness to typhoidin or similar glycerin extracts. We believe, furthermore, that sensitization to typhoidin persists for a longer period in rabbits than do serum antibodies, and that hypersensitiveness may exist even in the absence of demonstrable immune bodies.

EXPERIMENT-GROUP 3

RELATION OF THE SKIN REACTION TO THE CARRIER STATE

Cutaneous tests with tuberculin, sporotrichosin, streptothricin, etc., have thus far proved to be of inestimable value as a means of diagnosing an existing tubercular, sporotrichotic, or streptotrichotic infection (Claypole[10]). It is apparent from these facts that allergic tests have, thus far, only been employed successfully in chronic diseases, and their value in acute infectious diseases is, as previously stated, as yet undecided. Until very recently a positive skin reaction was considered an index of an existing infection and rarely as one of immunity. Römer[11] even went so far as to state that the intensity of the cutaneous reactions of a guinea-pig to tuberculin depends directly on the extent and advance of the tuberculous process. Krause[12] supported and enhanced these statements by additional observations, which we will consider later. The same conclusions were reached by us in some experiments with sporotrichosin on sporotrichotic rats, rabbits, and 1 human case.

[10] Arch. Int. Med., 1914, 14, p. 104.
[11] Beitr. z. Klin. f. Tuberk., 1909, 14, p. 1.
[12] Jour. Med. Research, 1916, 35, pp. 1, 21.

TABLE 3

RESULTS OF TESTS IN EXPERIMENT-GROUP 3

DATE OF TESTS: APRIL 10, 1916

Rabbit	Immunization	Result of Previous Test	Date of Reinjection (1916)	Serologic Tests April 10, 1916		Hours
				Agglutination	Complement-Fixation	
317	B. typhosus (Rawlings)	Feb. 3 See Table 3, Paper 1	Jan. 25	1:2000		16 24 48
332	B. typhosus 46	Feb. 3 See Table 3, Paper 1	March 2	1:2000 (1:800 Feb. 22)	0.003	16 24 48 72
335	B. typhosus 49	Feb. 23 See Table 6, Paper 1	March 2	1:10000 (1:600 Feb. 22)	A 0.05 * S 0.005	16 24 48 72
336	B. typhosus 50	Feb. 3 See Table 6, Paper 1	March 2	1:6000 to 1:8000 (1:2000 to 4000) (Feb. 22)	>0.003	16 24 48 72
337	B. typhosus 48	Feb. 23 See Table 5, Paper 1	March 2	1:4000 (1:4000 Jan. 14)	0.0005	16 24 48 72
353	B. typhosus 70		3 injections previous to Nov. 13, 1915	1:600 (1:6000 Dec. 28)	>0.003	16 24 48 72
355	B. typhosus 73	Feb. 23 See Table 6, Paper 1	March 3	(1:4000 Mar. 11) (1:1000 Feb. 22)	0.003	16 24 48 72
358	B. typhosus 78	Feb. 23 See Table 5, Paper 1	March 3	1:6000 (1:800 Feb. 22)	A 0.02 * S 0.0005	16 24 48 72
359	B. typhosus 79		March 3	1:6000 (1:800 Feb. 22)	A 0.02 * S 0.0005	Died during the test
361	B. typhosus 94	Feb. 23 See Table 6, Paper 1	March 3	1:6000 (1:4000 Feb. 21)	A 0.02 * S 0.0003	16 24 48 48
607 and 608	Controls repeatedly tested within 1½ months					
638	Control			1:10	0.05	16 24 48 72
639	Control			0	0.1 (50%)	16 24 48 72
698	Control			0	0.1 (50%)	16 24 48 72

A = antigen titration. S = serum titration.

TABLE 3—*Continued*

RESULTS OF TESTS IN EXPERIMENT-GROUP 3

DATE OF TESTS: APRIL 10, 1916

Typhoidin P. C.		Paraty-phoidin A 1:100	B. Coli 1:100	B. Paraty-phoidin B 1:100	Control Powder	Remarks
1:50	1:100					
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	
2.2 x 2.2	1.2 x 1.2	1.1 x 1.1	Red spot	Red spot	N. P.	Not a carrier
1.8 x 1.8	1.4 x 1.4	1.2 x 1.2	Red spot	Red spot	Red spot	
1.9 x 1.9	1.4 x 1.4	Red spot	1.8 x 1.8, delay	N. P.	Red spot	
1.8 x 1.8	1.6 x 1.6	N. P.	N. P.	N. P.	Red spot	Not a carrier
1.9 x 1.8	D. O.	N. P.	N. P.	N. P.	Red spot	
1 x 1	0.8 x 0.8	N. P.	N. P.	0.8 x 0.8	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
2.4 x 2.4, W. D.	1.2 x 1.2, W. D.	Red area	Red area	0.3 x 0.3	Red spot	April 19, 1916, B. typhosus in liver and gallbladder wall
3 x 3 W. D.	1.8 x 1.8, W. D.	1 x 1	1 x 1	Red spot	Red spot	
2.2 x 2.2, W. D.	1.3 x 1.3, W. D.	Red spot	Red spot	Red spot	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
2.4 x 2.4, W. D.	2 x 2, W. D.	1.5 x 1.5, R. I.	Diffuse red area	Diffuse red area	N. P.	April 10, 1916, B. typhosus in bile and gallbladder wall
3 x 3, W. D.	1.7 x 1.7, W. D.	1 x 1	1.8 x 1.8	N. P.	Red spot	
2.3 x 2.3, W. D.	1.6 x 1.6, W. D.	1.2 x 1.2	1.3 x 1.3	N. P.	Red spot	
Red area	Red spot	N. P.	Red area	N. P.	N. P.	
1.6 x 1.6	1.4 x 1.4	1.1 x 1.1	N. P	Red spot	N. P.	April 19, 1916, not a carrier
1.9 x 1.9	1.6 x 1.6	1.1 x 1.1	2.2 x 2.2	1.1 x 1.1	N. P.	
1.1 x 1.1	N. P.	N. P.	2.3 x 2.3	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	Red spot	Red spot	Red spot	Red spot	Red spot	Did not become carrier; bile sterile
Red spot	Red spot	Red spot	Diffuse red area	Red spot	Red spot	
1.3 x 1.3	Red spot	N. P.	2 x 2	N. P.	N. P.	
Red spot	N. P.	N. P.	N. P.	N. P.	N. P.	
Red area	Diffuse red area	Red spot	Red spot	Red spot	Red spot	April 22, 1916, B. typhosus in gallbladder wall
2.4 x 2.4, W. D.	1.8 x 1.8, W. D.	Red spot	Red spot	Red spot	Red spot	
0.8 x 0.8, R. I.	Red spot	N. P.	N. P.	N. P.	N. P.	
Red spot	N. P.	N. P.	N. P.	N. P.	N. P.	
1 x 1	1.2 x 1.6	0.6 x 0.6	1.4 x 1.4	1.8 x 1.8	Red spot	Emaciated and clinically sick; B. typhosus in bile
1.5 x 1.5, W. D., very good	1.5 x 1.5, W. D.	0.8 x 0.8	1.4 x 1.4	1.1 x 1.1	Red spot	
0.8 x 0.8 Small nodule	0.8 x 0.8 Red spot	N. P. N. P.	1 x 1 Red spot	Nodule Red spot	N. P. N. P.	
						April 11, 1916, B. typhosus in bile and gallbladder wall
2.2 x 2.2, W. D.	2.1 x 2.1, W. D.	1.2 x 1.2	1.5 x 1.5	1.5 x 1.5	1.5 x 1.5	Emaciated and clinically sick. April 14, 1916, B. typhosus in bile
2.4 x 2.4, W. D.	2 x 2, W. D.	1.1 x 1.1	2 x 2	1.3 x 1.3	Red area	
1.8 x 1.8, W. D.	1.3 x 1.3, W. D.	Red spot	1.8 x 1.8	1.8 x 1.8	1 x 1	
Red nodule	Red spot	N. P.	Red area	Red spot	Red area	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	Sl. swelling	N. P.	N. P.	
N. P.	N. P.	N. P.	0.5 x 0.5	N. P.	N. P.	
N. P.	N. P.	N. P.	Red spot	N. P.	N. P.	
N. P.	N. P.	N. P.	Red spot	N. P.	N. P.	
N. P.	N. P.	N. P.	0.8 x 0.8	N. P.	N. P.	
N. P.	N. P.	N. P.	0.4 x 0.4	N. P.	N. P.	
N. P	N. P.	N. P.	Red spot	N. P.	N. P.	

The scope of our investigation therefore called for a study of cutaneous hypersensitiveness in rabbits which harbored the typhoid bacilli somewhere in the body. From previous experiments we had derived practice in producing 'gallbladder' carriers by the intravenous injection of large doses of living typhoid germs into immune rabbits. For numerous reasons it was impossible to ascertain with certainty when the animals began to harbor the organisms in the liver or gallbladder (stool cultures, even in good carriers, being often negative), until after the skin test had been completed. The striking results which we obtained in Experiment-group 3 were therefore observed at a time when we had no knowledge of the degree of typhoid infection.

Nine rabbits (332, 335, 336, 337, 355, 358, 359, 387, 361) possessing a basic immunity, had been inoculated intravenously on January 25, March 2, and March 3, respectively, with one-half rabbit-blood agar slant of the same typhoid strain which had been used for immunization. Previous to this test inoculation on Feb. 2 or 23, most of these rabbits had received an intracutaneous typhoidin test. The results of this test are shown in Tables 3 and 5 of our 1st paper. One rabbit (353) which had thus far received only 3 small injections of a killed typhoid culture previous to Nov. 13, 1915, together with 3 normal rabbits (638, 639, and 698) and 2 rabbits (607 and 608) which had been tested intracutaneously on a previous occasion, served as controls.

Most of these rabbits were killed and examined in from 2 to 16 days after the test, and it was found that Rabbits 335, 336, 355, 358, 359, and 361 were carriers of the bacilli in gallbladder or liver, and therefore were infected at the time of the typhoidin test. Three rabbits (358, 359, and 361) were considerably emaciated and anemic at the time of the test, one (359) even died during the experiment. It was very fortunate that we incorporated in the experiment some rabbits which had undergone the same treatment, but which had not developed a carrier condition. The immune bodies were present in the animals in similar or even larger amounts than in the carriers, and these were therefore most suitable for work on the problem of this experiment.

The test preparation used in these instances was a typhoidin obtained from a 41-day-old glycerin-potato-broth culture (Strain Olsen) which after extraction and concentration had been filtered through paper and then precipitated. It had an antigenic value of 5 e.u. per milligram, and in many other experiments proved to be an excellent preparation. The paratyphoidin-A and -B and the B.-coli extracts were prepared in the same manner. The granular powders were readily dissolved in carbolized salt solution, and these made perfectly clear solutions.

The results of the test are summarized in Table 3.

We consider this experiment to be by far the most interesting of our series, in the first place, on account of the low primary toxicity of the preparations, and secondly on account of the marked specificity of the reactions. With the exception of the B.-coli extract, neither preparation gave the slightest reaction in the control rabbits as far as they were not already sensitized by cutaneous tests. Furthermore, the

reactions were well defined in the immunized and infected animals. Some of the reactions were so striking that we could predict the carrier condition, in advance, from the intensity and induration of the areolae. The 24-hour readings (which show, as a rule, greater intensity than the 48-hour readings) were particularly prominent in Rabbits 335, 336, 355, and 361, with 0.02 gm. of typhoidin. In Rabbit 358 and to a certain extent in 361, the response to typhoidin was influenced by the clinical condition of the animal. It is known that during emaciation and anemia, cutaneous tests are frequently diminished in intensity. In most of the clinically healthy rabbits which, as previously stated, later proved to be typhoid carriers, the reaction persisted in a proportionately high degree of intensity for 48 hours. Only Rabbits 355 and 358 were exceptions. In 1 animal (358) this was probably due to an emaciated condition, and, in the others, to some unknown individual factors.

The highly immune rabbits (317, 332, 337) on the other hand, gave smaller, less indurated areolae, which did not persist to the 48th hour. In the quantity of 0.001 gm. the typhoidin P.C.—as was to be expected from its low antigenic value—was not very active. It gave, however, large and indurated areolae in the infected rabbits, and its action as an anaphylactogen was not much impaired apparently, even when the antigenic value promised a different result. The most striking differences between infected and immunized rabbits with a dilution of 1 : 100 or 0.001 gm. per test were noted at the 24-hour readings.

Rabbit 353, not well immunized, responded poorly to typhoidin. At the 48-hour reading a small red areola with very little induration was present. This negative result was in marked contrast with all the gradations observed in the immune and infected rabbits. From Table 3 it also is apparent how difficult it would be to judge the degree of immunity and the degree of sensitization of a rabbit to B. typhosus, from the amount of serum immune bodies. For example: Rabbit 336 showed the same amount of complement-fixing antibodies as 355, yet their skin reactions are different; or, 317 and 335 with the same amount of complement-fixing bodies have an entirely different degree of hypersensitiveness. These observations only confirm our conclusion that no relationship exists between serum immune bodies and the cutaneous response of a rabbit to typhoidin. Some of the carriers and some of the immune rabbits responded to paratyphoid-A and -B and B.-coli extracts in quantity of 0.001 gm. The only additional observation with these nonspecific extracts worthy of emphasis is that the

emaciated and sick carriers reacted uniformly to all the extracts. As we know from previous discussions, general hypersensitiveness in some animals is frequently nonspecific as the result of disease or infection.

The results with Group 3 show perfectly that it is possible to detect the carrier state in rabbits by means of the typhoidin test as long as the carrier state is produced by means of intravenous inoculations. Some preliminary experiments on rabbits which had been infected by direct gallbladder-injections showed that the skin reactions are not always as prominent as those noted in Experiment 3 in the rabbits in which the conditions of a human carrier had been imitated as closely as possible. We are at present carefully studying this condition and hope to report some of our observations in the near future.

This experiment also supports our contention that specific cutaneous hypersensitiveness to typhoidin is most marked in an existing typhoid infection of the rabbit. Apparently cutaneous hypersensitiveness in typhoid rabbits varies also directly with the extent and intensity of the disease, as is true of tuberculosis in guinea-pigs. In Rabbit 355 the inflammatory process in the gallbladder was already healing at the time of the test, and therefore the hypersensitiveness was diminished in comparison with that noted in rabbits with flourishing infections. The once-acquired sensitiveness (by infection or artificial means) is probably never entirely lost, tho it may exhibit variations due to factors thus far unknown which we fear cannot be determined experimentally on rabbits. In our experiments intercurrent infections due to B. bipolaris-septicus, for example, were capable of removing the hypersensitiveness entirely. We have thus far been unable to keep infected rabbits for a sufficiently long period (from 1 to 2 years) to test their cutaneous sensitiveness until it entirely disappears.

It would be of the greatest interest to study some human typhoid-carriers and to compare their cutaneous hypersensitiveness to typhoidin with that found in persons infected, reconvalescent, immune, or artificially immunized against typhoid. In using pure typhoprotein and glycerin extracts applied in various dilutions, the problem of cutaneous hypersensitiveness to typhoprotein and its relation to immunity would probably be more satisfactorily solved than has hitherto been the case.

DISCUSSION

The well-known natural immunity of rabbits to an infection with the typhoid bacillus, and the limited possibility of reproducing in this type of animal a disease anatomically and clinically similar to typhoid

fever in man, naturally do not permit of a direct application of our observations to problems of clinical and preventive medicine. And again, the knowledge concerning the mechanism of typhoid immunity in man and animals is so very limited and the present experimental means so crude, that we have to content ourselves with some suggestions which developed from our laboratory observations.

It should be remembered that, aside from morbidity statistics and a few experiments on anthropoid apes, we do not as yet possess means by which we can determine, with certainty, an existing natural or acquired immunity of man or animal against typhoid. Some investigators think that the body fluids, others that the cells of the blood and lymph, or of the tissues most apt to get in contact with the typhoid bacillus, participate in suppressing the fatal multiplication of the invading microorganisms. The conception of a cellular immunity has recently found more and more adherents. The studies of von Wassermann and Sommerfield[13] have added considerable support to this conception. These workers found that in mice the well-balanced natural local immunity of the intestinal canal to typhoid bacilli could readily be broken by intercurrent infections or intoxications. These facts lend considerable support to the epidemiologic observations, recently made, that typhoid immunity in man is, in all probability, only relative and that reinfections, with little or no symptoms, are not as rare an occurrence as has been commonly thought to be the case. With typhoid strains highly pathogenic for rabbits, we attempted similar experiments but have thus far only negative results to record.

Our attempts to reproduce in rabbits the mode of typhoid infection in man, by successive passages of the typhoid bacillus through the gallbladders of the animals, are promising in many respects. Until we have succeeded, however, in producing in rabbits a typical intestinal typhoid infection, our methods, which — as already pointed out — are by no means analogous to the conditions in man, have very little value for the determination of the immunizing properties of a vaccine preparation and, in our particular case, for the study of the relation of immunity to the skin test.

The defensive activity of the rabbit following intravenous injection of living typhoid organisms, depends probably to a large extent on the serum immune bodies already present or ready to be mobilized (Bull[14]). The absence of a gallbladder carrier state in immunized rabbits subse-

[13] Med. Klin. 1915, 11, p. 1307.
[14] Jour. Exper. Med., 1916, 23, p. 419; 24, pp. 7, 25.

quent to injection of large amounts of living bacteria of the colon-typhoid-paratyphoid group can not, in our experience, be used as positive evidence of protection. Many factors which we are at present investigating are probably concerned in the pathogenesis of the carrier state, but it is already quite apparent that immunized rabbits develop gallbladder lesions more readily than do normal ones. In having established this fact we were naturally deprived of the most important criterion by which we could judge the immunity of our test animals.

In an endeavor to produce more conclusive results, we employed the fowl-typhoid bacillus as a test organism. The work of Theobald Smith and Ten Broeck,[6] and our own, has shown that a typhoid-immune rabbit is protected against the toxin of the fowl-typhoid bacillus. Inasmuch as cutaneous hypersensitiveness to fowl typhoidin was demonstrable, we hoped to be able to find some relationship between the degree of the reaction and the resistance of the animals to a subsequent injection of living fowl-typhoid organisms. No parallelisms between skin reaction and resistance to infection could be detected, however. These observations are, in many respects, analogous to an example cited by Nichols.[15] Persons immunized in a prophylactic manner against typhoid will give skin reactions with Paratyphoidin-A and yet possess no immunity to a spontaneous infection.

Whatever method one might choose, therefore, in testing the resistance of a rabbit, one would always produce unnatural conditions which, even if they were conclusive in every respect, would have no bearing on the problem in man. Our findings which show that there does not exist any demonstrable parallelism between cutaneous hypersensitiveness even to typhoidin and the resistance to a subsequent infection, apply therefore only to the rabbit. It is not unlikely that cutaneous hypersensitiveness to typhoidin in man is open to an interpretation different from that which we are able to formulate from our animal experiments.

The fact that infected rabbits react with typhoidin more intensively than immunized rabbits, is quite in accord with the general observations on the tuberculin reaction. Tuberculin hypersensitiveness is a sign of a tubercular infection. For typhoid fever in man, observations are on hand of hypersensitiveness during the acute attack of the disease, but no records could be found of skin tests in carriers. It is to be expected that carriers either give a very marked skin reaction

[15] Jour. Exper. Med., 1915, 22, p. 780.

analogous to our results, or fail to respond on account of absence of antibodies. In case future tests show that the skin reactions in carriers are always positive, we would be justified in concluding that the typhoidin test is a suitable diagnostic agent for the detection of an occult typhoid infection.

This conception is naturally open to criticism, because a gallbladder carrier state develops, as a rule, on the basis of a progressive or already existing immunity and a carrier has a fair degree of resistance to a superinfection even when not always protected against an auto-infection.

Many careful experiments and studies on man must be undertaken before we are prepared to render a final judgment on the merits of the typhoidin test.

Our experiments suggest, however, that careful quantitative tests with pure typhoprotein should be made on carriers of human typhoid. A simple test like the typhoidin test, if it should develop that this test does detect carriers, would be a valuable weapon in the hands of the health officer in the eradication of typhoid fever from communities in which the infection is apt to be perpetuated in this manner.

CONCLUSIONS

A positive typhoidin skin reaction in a rabbit does not indicate that this animal will resist a subsequent intravenous injection of living typhoid bacilli, or that the animal is so protected that it will not become a chronic carrier of bacilli in gallbladder or liver. And again, a marked hypersensitiveness of a typhoid-immune rabbit to fowl typhoidin does not indicate the presence of a resistance to an infection with the same organisms.

No definite relationship exists between agglutinins and complement-fixing antibodies and cutaneous hypersensitiveness to typhoidin and similar extracts. Allergy with bacterial proteins may be demonstrated in rabbits even in the absence of demonstrable immune bodies.

Cutaneous hypersensitiveness to typhoidin is most marked in rabbits infected with typhoid bacilli. Carriers of bacilli in gallbladder and liver develop skin reactions which apparently vary directly with the degree of the inflammatory process in the organs.